Detection of High Levels of Bromine in Vegetables Using X-Ray Fluorescence Spectrometry

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We examined the contents of different vegetables using X-ray fluorescence spectrometry and found extremely high levels of bromine (Br) [1000–16200 ppm (dry weight)] in some vegetables from an Osaka market. Examination using a bromide ion-selective electrode suggested that the bromine existed mainly as bromide ion. Since this bromine probably originated from fumigation with methyl bromide, this result suggests that fumigation methods may need to be regulated. Fifty milligrams of bromine [aceptable daily intake (ADI)/50 kg] was found in only 3.1 g (dry weight) of *komatsuna* (Br: 16200 ppm). These results suggest that vegetables that contain potentially harmful levels of bromide may be found in Japanese markets.

Key words —— bromine, X-ray fluorescence spectrometry, high content, vegetable

INTRODUCTION

Bromine (Br) is one of the most abundant and ubiquitous of the recognized trace elements in the biosphere. It has not been conclusively shown to perform any essential function in plants, microorganisms, or animals.[1] Food contains inorganic bromide at levels that depend on the type of foodstuff. When crops have been grown in soil fumigated with methyl bromide, they contain much more inorganic bromide than those raised in unfumigated soil.[2,3] Some inorganic bromide in crops is considered to be a breakdown product of brominated fumigants that are used to control pests.[3–5] Residual bromide levels are usually higher in such food than in unfumigated products.[6] Many researchers have focused on inorganic bromide due to the high daily intake from food and drinking water.

We have been studying major and trace elements in food using both X-ray fluorescence spectrometry and inductively coupled plasma mass spectrometry. We found abnormally high levels of bromine in vegetables from an Osaka market using X-ray fluorescence spectrometry. These vegetables could be harmful to humans. In this report, we describe the bromine contents of some vegetables, the urgent need for concern regarding vegetables with high levels of bromine, and the need for further investigation of the actual source of this element.

MATERIALS AND METHODS

Materials —— Vegetable samples (24 different vegetables, 97 specimens) were purchased in Japanese markets (mainly Osaka Prefecture) from September 2000 to April 2004. All reagents were of the highest quality available.

X-Ray Fluorescence Analysis —— Dried vegetable samples were powdered in a blender (TOP, WB-1) and then placed in a silica gel desiccator for more than 24 hr. Each powdered sample (450 mg) was pressed into a pellet of 2.0 cm in diameter at 10 tons of pressure. X-ray measurements were performed on the pellet samples with a wavelength-dispersive X-ray fluorescence spectrometer (Rigaku ZSX100s) equipped with a rhodium anode X-ray tube. Element contents were analyzed using the fundamental parameter (FP) method with several standard samples, according to the manufacturer's protocol.

Analysis of Bromide Ion —— One gram of each vegetable powder (high bromine content) was extracted with water (30 and 10-ml portions) at 50°C. The combined aqueous solution was adjusted with water to a volume of 50 ml. The bromide ion concentration was measured using a bromide ion-selective electrode (Horiba ion meter F-23 equipped with an 8005 electrode).

RESULTS AND DISCUSSION

Figure 1 shows the X-ray fluorescence spectra for a typical sample containing a high bromine level and a normal sample of *komatsuna*. Qualitative analysis was easily performed based on the peak

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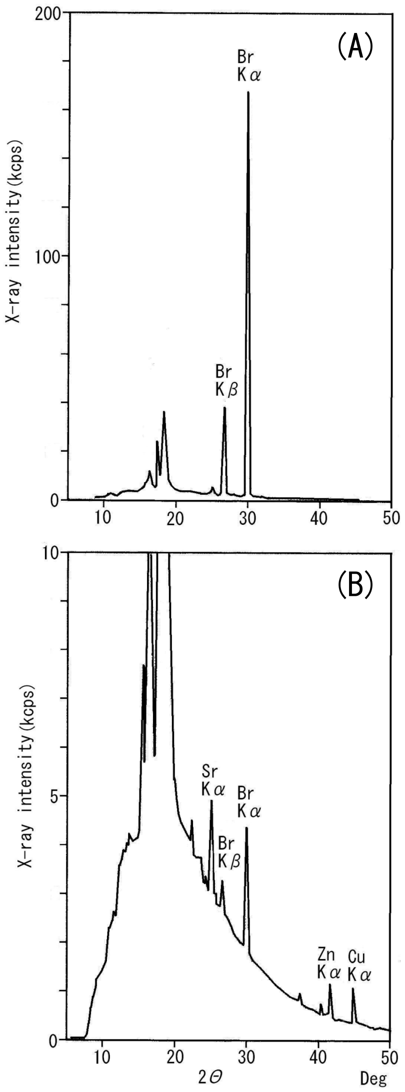

Fig. 1. X-Ray Fluorescence Spectra of Two Samples of the Vegetable *Komatsuna*

(A), *Komatsuna* containing high bromine level. (B), *Komatsuna* containing low bromine level.

position (diffraction angle, 2θ) of the characteristic X-rays generated from sample materials. The characteristic X-rays (Kα and Kβ) of bromine appeared at specific positions in the upper spectrum (A, *komatsuna* with high bromine content) with extremely strong intensity, compared with that of the sample with lower levels (B, normal *komatsuna*) (Fig. 1). Two of the seven *komatsuna* samples tested had a high bromine content and the others a low content. A high bromine content was also detected in other vegetables, such as Chinese cabbage, *shungiku* (corn marigold), Welsh onion, *etc*. As in *komatsuna*, samples of other vegetable had both high and low bromine contents.

Table 1. Recovery Rates of Total Bromine from Vegetable (Cabbage)

Added$^{a)}$	Recovery (%)$^{b)}$	C.V. (%)
2000	99.3	0.9
1000	104.5	1.2
500	98.4	1.1
100	97.1	0.9
50	97.2	1.2

C.V., coefficient of variation. *a*) Amounts are expressed as ppm (dry weight) of cabbage. *b*) Values are means ($n = 5$).

Table 2. Determination of Total Bromine in Standard Reference Materials Using the Proposed Method

Sample	Total Bromine (ppm)$^{a)}$
Tomato leaves (NBS 1573)	22.7 ± 0.6 (26)$^{b)}$
Tomato leaves (NIST 1573a)	1286 ± 14 (1300)$^{b)}$
Sea lettuce (BCR 279)	381 ± 5 (346)$^{b)}$

a) Mean ± standard deviation based on three trials. *b*) Reference values.

Quantitative analysis requires correction for preliminary and secondary effects of the sample matrix. These effects can be represented by very complex equations. Most of the vegetables contained light elements such as H, C, O, and N. The principal constituent in a plant sample is polysaccharide, such as cellulose ($C_6H_{10}O_5$)n. Starch has the same general formula, and other sugars have a similar composition. The X-ray fluorescence technique should be applicable to nearly all elements heavier than sodium. Since the matrix effects could be calculated by assuming that the residual composition is cellulose, quantitative analysis using the FP method is possible.[7,8)]

Table 1 shows the recovery rates of total bromine added to standard vegetables (cabbage, Br: 6.3 ppm). Recovery rates of 50, 100, 500, 1000, 2000 ppm were between 97.1 and 104.5%.

To confirm the accuracy of this method, samples with known bromine concentrations were measured. Table 2 shows the analytical results of total bromine in standard reference materials using this method. The analytical results were in good agreement with the certified values, with an accuracy of 13% or better for a wide range of bromine concentrations. Thus this method appears to be suitable for the determination of bromine in vegetables.

Table 3 shows the analytical results (total bromine) for various vegetables by this X-ray fluores-

Table 3. Total Bromine Contents in Some Vegetables

Samples	No. of samples	Total bromine content (ppm)	
		Analytical values	Average
Tsurumurasaki	2	23, 23	23
Spinach	12	5, 8, 10, 11, 14, 14, 15, 16, 31, 50, 68, 108	29
Shungiku	7	35, 64, 85, 128, 3330, 6330, 6990	2420
Lettuce	14	3, 3, 4, 5, 5, 6, 11, 11, 15, 17, 21, 39, 40, 53	17
Saladana	3	4, 6, 9	6
Chinese cabbage	2	23, 3650	1840
Cabbage	5	4, 4, 7, 8, 13	7
Mibuna	2	3, 10	7
Shirona	5	7, 26, 120, 1150, 2460	752
Komatsuna	7	11, 18, 33, 55, 67, 5260, 16200	3090
Chingensai	3	6, 24, 54	28
Kaiware	6	2, 4, 8, 8, 17, 22	10
Welsh onion	6	7, 11, 13, 17, 17, 1820	314
Onion	1	3	3
Nira	3	85, 106, 115	102
Garlic sprout	1	3	3
Toumyou	1	10	10
Tea	4	9, 11, 15, 15	13
Jew's mallow	2	14, 250	132
Shiitake mushroom	3	1, 1, 3	2
Parsley	5	6, 11, 13, 21, 22	15
Japanese Honewort	1	47	47
Water Dropwort	1	11	11
Coriander	1	15	15

Tsurumurasaki, *Basella rubra* var. *alba*; *Shungiku Chrysanthemum coronarium* var. *spatiosum*; *Mibuna*, *Brassica campestris*; *Komatsuna*, *Brassica campestris* subsp. *napus* var. *komatsuna*; *Chingensai*, *Brassica chinensis*; *Kaiware*, *Raphanus sativum* var. *acanthiformis*; *Nira*, *Allium tuberosum*.

cence spectrometry. Bromine was detected in all of the vegetable samples examined. In vegetables with high bromine contents (more than 1000 ppm), quantitative analysis showed 16200 and 5260 ppm in *komatsuna*, 6990 and 6330 ppm for *shungiku*, 3650 ppm for Chinese cabbage, 2460 and 1150 ppm for *shirona*, and 1820 ppm for Welsh onion. The *shungiku*, *shirona*, and *komatsuna* samples were grown in Osaka Prefecture. The Chinese cabbage and Welsh onion were from Nagano and Kochi Prefectures, respectively. With X-ray florescence spectrometry, total bromine can be detected, but the chemical form can not be determined. On the other hand, the ion-selective electrode method can be used to clarify the bromide ion concentration. The bromide ion contents of high-bromine vegetables are shown in Table 4. The similarity of the concentration of total bromine and bromide ion shows that almost all of the bromine in these vegetables exists as bromide ion. Slightly higher values of bromide ion can be accounted for by the positive interference from coexisting chloride ion in the samples of *komatsuna*. These results indicate an abnormally high content of bromine (as bromide ion) in some vegetables.

Table 4. Bromide Ion Contents in Some Vegetables

Sample	Total bromine (X-ray fluorescence)	Bromide ion (Br ion electrode)
Shungiku-1	3330	2890
Shungiku-2	6990	6550
Shirona-1	2460	2210
Shirona-2	1150	1150
Komatsuna-1	5260	5530
Komatsuna-2	16200	17900
Chinese cabbage	3650	3150

When bromide is administered to humans, rash, bromism, central nervous system depression, mental deterioration, and acneform skin eruptions may occur.[9] Since excess bromine uptake [acceptable daily intake (ADI): 1 mg/kg body weight/day) may cause poor health, residual standard values have been determined for 44 crops in Japan, but not for the vegetables tested in this study.[9] Among the bromine contents that have been reported in foodstuffs so far, bromine levels of more than 100 ppm (fresh weight) are very rare. For example, only four samples [Welsh onion, 170 ppm (fresh weight); *shungiku*, 139 and 106 ppm (fresh weight); pumpkin, 103 ppm (fresh weight)] were found among 622 samples (52 different crops) from a Hyogo market using electron capture detector-gas chromatography.[10] The estimated daily intake of bromide from food has been calculated to be very low and safe for adults, even if the highest value (170 ppm) is used for the calculation. However, in this study, 50 mg of bromine (ADI/50 kg) corresponds to only 3.1 g of *komatsuna* (16200 ppm). These results suggest that there is a high likelihood of unhealthy bromine intake. Since the bromine in vegetables is known to be due to fumigation with methyl bromide, these high bromine contents probably arise from the fumigation of greenhouses and/or soil. Yuita reported that the residual bromine contents in vegetables grown in a fumigated greenhouse were more than 10-fold higher than the contents in vegetables grown in an unfumigated greenhouse, and that the amount of fumigant used should be decreased.[11] The bromine contents of produce from Japanese markets should be carefully examined to obtain accurate information. Furthermore, a response to this apparent problem is urgently required, *e.g.*, a criterion for residual bromine contents should be established and the method used for fumigation of these types of vegetables may need to be regulated.

This is the first report to show that some vegetables that contain bromine at very high, and possibly harmful, levels can currently be found at Japanese produce markets.

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